ZOOLOGIA 35: e12874
ISSN 1984-4689 (online)
zoologia.pensoft.net

RESEARCH ARTICLE

On the habitat use of the Neotropical whip spider *Charinus asturius* (Arachnida: Amblypygi)

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http://zoobank.org/51469941-5834-4AA2-A1DB-A424F0377392

ABSTRACT. The non-random occupation of habitats is termed habitat selection. Some species of whip spiders select trees with burrows at their base, while others use substrates such as rocks. Here, we investigated the habitat use by *Charinus asturius* Pinto-da-Rocha, Machado & Weygoldt, 2002, an endemic species of Ilhabela Island in Brazil. We found that *C. asturius* is more likely to be found under rocks that cover larger areas of substrate. Our results also suggest the existence of territorialism in *C. asturius* and show that *C. asturius* adults may be found again on the same rock a week later. Additionally, our data show that *C. asturius* is present in a greater area of Ilhabela than previously documented.

KEY WORDS. Amblypygids, natural history, shelter.

INTRODUCTION

Habitat selection is a process by which individuals preferentially occupy a set of available habitats non-randomly (Morris 2003). A common behavior of many arthropods is sheltering, which can provide microclimatic advantages (Cloudsley-Thompson 1962) and defense against predators (Edmunds 1974). Among arachnids, scorpions may select a deeper leaf litter layer in the dry season and a shallower one in the wet season (Lira et al. 2013); some spiders may show preference for vertical shelters rather than horizontal ones (Vetter and Rust 2008); and amblypygids may select trees with burrows at their bases (Porto and Peixoto 2013).

The order Amblypygi (sometimes called whip spiders) comprises a little more than 200 species (Miranda et al. 2016). They have raptorial pedipalps bearing spines that are used to strike prey (Weygoldt 2000, Santer and Hebets 2009). The first pair of legs, popularly called whips, are antenniform and used as feelers (Weygoldt 2000, Santer and Hebets 2011). The whip spiders' laterigrade legs and flattened body enable them to shelter in crevices, under tree bark and fissures (Weygoldt 2000). They are usually territorial and forage at night (Weygoldt 2000). It is also known that some whip spiders can exhibit shelter fidelity at least in a scale of days (Hebets et al. 2014, Bingman et al. 2017).

The majority of field studies on whip spiders focused on species that use tree trunks to shelter during the day, but little is known about the natural history of whip spiders that use different substrates as shelters, for example rocks (Chapin and Hebets 2016 and references therein). Furthermore, there is also a phylogenetic bias, since most of the field studies with whip spiders have been carried out with recently evolved lineages, mainly belonging to the family Phrynidae (Weygoldt 2000, Chapin and Hebets 2016 and references therein). Except for reproductive biology (Weygoldt 2008a, b), little attention has been given to the behavior of early branching lineages for example the family Charinidae (but see Pinto-da-Rocha et al. 2002, Miranda et al. 2016).

Charinus Simon, 1892 is represented by relatively small species that are almost worldwide distributed (Weygoldt 2000) and most species occur in Brazil (Giupponi and Miranda 2016). Regarding the microhabitat, they may inhabit caves or epigean habitats, sheltering in bromeliads, under rocks and in leaf litter. However, most data concerning the natural history of this genus come from species description papers (Pinto-da-Rocha et al. 2002, Jocque and Giupponi 2012, Vasconcelos et al. 2013, 2014, Giupponi and Miranda 2016). Thus, there is a lack of field studies concerning the habitat use of *Charinus*' species.

Charinus asturius Pinto-da-Rocha, Machado & Weygoldt, 2012 is endemic to Ilhabela Island, Brazil. This species is sexually dimorphic, with adult males having larger pedipalps than females (Pinto-da-Rocha et al. 2002). Being nocturnal, individuals of *C. asturius* can be found sheltering under rocks during the day (Pinto-da-Rocha et al. 2002). Previous qualitative data suggests that they rest preferentially on rocks (Pinto-da-Rocha et al. 2002). Herein we quantitatively compared the occurrence of *C. asturius* between rocks and the adjacent forest floor. With respect to shelter features, larger rocks can provide more stable temperature and humidity and can offer a better protection against predators (Dean and Turner 1991). Therefore, we predicted that the larger the rock, the higher the probability of finding *C. asturius*. We also evaluated whether *C. asturius* exhibits shelter fidelity and finally looked for *C. asturius* in areas of the island with no registers for this species. Given that whip spiders can defend their territory (Chapin and Hill-Lindsay 2016, but see Rayor and Taylor 2006), they are not expected to stay close to each other except for sexual reasons. We therefore measured the distance between conspecifics, to gather preliminary information about territorial behavior in this species.

MATERIAL AND METHODS

We conducted this study in the Pacuíba Mount, an area of Atlantic rainforest in the North of Ilhabela Island, Brazil, in the warm and rainy season – Feb. 22–28, 2016 (23°42.828'S, 045°19.045'W). We collected the data of all steps (see below) during the day (9:00 am to 5:30 pm). We tentatively determined the sex of the *C. asturius* individuals based on the sexual dimorphic features of this species (i.e. relative pedipalp width).

We sampled 15 quadrants (5 x 5 m) only once looking for individuals of *C. asturius*. We distributed the quadrants in areas with greater number of rocks, since a previous study reported that *C. asturius* use them as shelters (Pinto-da-Rocha et al. 2002). The quadrants were at least 10 m apart from each other. The elevation of sampled area ranged from 72–214 m.

We compared the occurrence of individuals of *C. asturius* between rocks and forest floor. For each rock surveyed we also examined an adjacent forest floor area of similar dimensions (one exception occurred in one of the quadrants, which was almost totally covered by rocks). We excluded this quadrant from the analysis that compared the occurrence of specimens under rocks and in adjacent areas (see below), so that the sample size here was 257 rocks. We sampled the forest floor by hand removing the leaf litter. The starting point of the survey was alternated between rocks and adjacent area. We chose this procedure to avoid any biases resulting from animals running from one area to another. We compared the occurrence of *C. asturius* on rocks and adjacent areas with a Chi-square test.

To test the prediction that the larger the rock, the higher the probability of finding whip spiders, we lifted all rocks (N = 291) inside each quadrant mentioned above, including the quadrant that was excluded in the previous analysis (except the rocks that were smaller than 10 cm in length and the rocks that we were physically unable to move). We measured the maximum length and maximum width for rocks that had their whole undersurface extension close to substrate (= less than 0.5 cm above the substrate). In the case of rocks that had part of their undersurface further than 0.5 cm from the substrate, we measured the length and width of the parts that were less than 0.5 cm above the substrate. We did this because the area close to the substrate has probably more influence on the microclimatic conditions and protection from predators than the total rock size *per se*. We calculated the area of the rock that was close to the substrate with the ellipse equation ($A = r1 \times r2 \times pi$). Then, we performed a logistic regression with the area of the ellipse as an independent variable and the presence and absence of whip spiders as the dependent variable.

When we found individuals of *C. asturius* under rocks, we marked the rocks with an adhesive tape with the identity of the whip spiders on it. After surveying all rocks and adjacent areas in the quadrant, we measured the distance between whip spiders and the closest conspecific inside the quadrant, based on the distance between the centers of the rocks where the animals were found. Since we have found some juveniles and adults under the same rock (see below), we assumed that there are no territorial disputes between adults and juveniles. Thus, we only measured the distance between adults to other adults, and the distance between juveniles to other juveniles.

We also investigated whether *C. asturius* may use the same rock during several consecutive days. We conducted manipulations at the same location described above, from February 21st to 28th 2016. During the first day (between 9:00 am to 5:00 pm, when the animals are expected to be at rest) we lifted random rocks to find individuals of *C. asturius*. After finding the whip spiders, we individually marked 14 specimens of *C. asturius* on 13 rocks (in one case there were a male and a female below the same rock). Then, we carefully put the whip spiders back on the rocks where they were previously caught. After that, we marked the rocks where they were found. We marked both the whip spiders' legs and rocks with colored fabric paint Acrilex. After 7 days we came back to the same area during the day (between 9:00 am to 5:00 pm) and lifted the marked rocks again to verify if the individuals were there. If the individual was not on the original

rock, we surveyed rocks of the same conglomerate (which we defined as rocks within a radius of 1.5 m from the rock where the animal was first found).

We conducted an exploratory search in other areas of the Island because this species was previously recorded from only a single location (Pacuíba Mount).We searched for whip spiders (October 2015) at the Jabaquara trail – North of the Island, (23°43.833'S, 045°17.268'W); Água Branca trail (23°50.479'S, 045°21.631'W) – Center of the Island; and Sepituba trail (23°43.833'S, 045°19.053'W) – South of the Island. The vouchers were deposited at the Museum of Zoology of the University of São Paulo (MZSP 71001).

RESULTS

We have found 62 individuals of *C. asturius* inside the quadrant's area, always on the undersurface of the rocks. None were found on the forest floor adjacent to rocks (rocks vs. adjacent forest floor $\chi^2 = 51.046$, df = 1, p = 0.000).

The probability of finding whip spiders enhances as the area of the rocks close to the substrate increases ($\chi^2 = -13.13$, df = 1, p = 0.00029, Fig. 1). It is worthy highlight that this result is not biased as consequence of sampling a larger area (i.e. the total area of all rocks) in the group with whip spiders. We actually sampled a larger total area in the group without whip spiders: the sum of the elliptic area close to the substrate of all rocks was 34,004.43 cm^2 where individuals were found (n = 62) and was 81,243.3 cm^2 for the rocks without individuals (n = 229).

The median distance between conspecifics (Fig. 2) were: two females (n = 10): 54.5 cm, min = 11, max = 225; male and a female (n = 6): 129.5 cm, min = 23, max = 299; between juveniles (n = 7): 23.0 cm, min = 16, max = 76. In the only case that we found 2 males inside the same quadrant the distance between them was 75 cm. No two adults (of the same sex) were found on the same rock in this survey.

With respect of shelter use, we encountered seven out of 14 whip spiders on the same rock where they were sheltering one week before (two of them were a male and a female on the same rock). We also found two individuals not on the same rock, but on the same conglomerate of rocks. We did not find the remaining five individuals.

Concerning the species distribution, we encountered *C. asturius* both in the Jabaquara trail and Água Branca trail but not in Sepituba trail. We found ovigerous females in every field trip (February 2016 and October 2015). We have found a *Loxosceles* Heineken & Lowe, 1832 spider sharing the same rock of whip spiders in two different locations (Jabaquara trail and Pacuíba Mount). In the latter we also encountered a dead female of *C. asturius* under the same rock of a recluse spider (Fig. 3). The spider was not eating it.

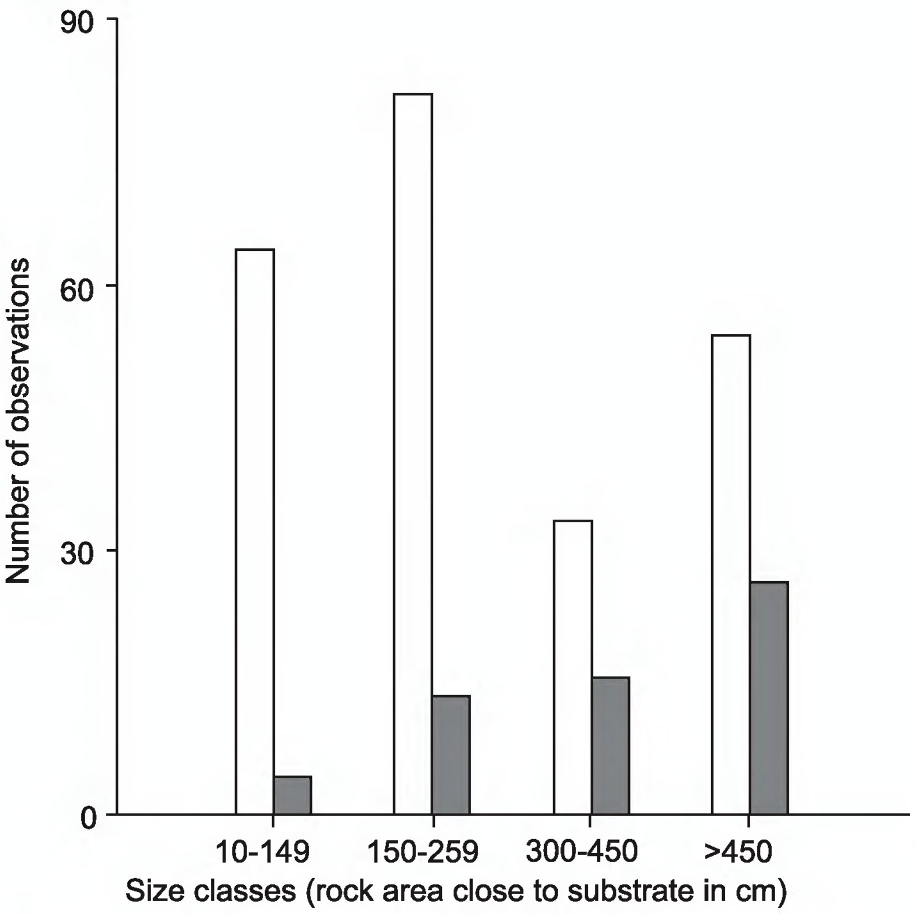

Figure 1. Number of rocks observed without (white bars) and with (black bars) individuals of *Charinus asturius* per classes of sizes regarding the area (cm) close to the substrate. For the sake of clarity the size classes were divided 150cm^2 ranges, except for the class (above 450cm^2) in which the median was 670.4 cm^2 and the values range from (459.2-2882.5 cm^2).

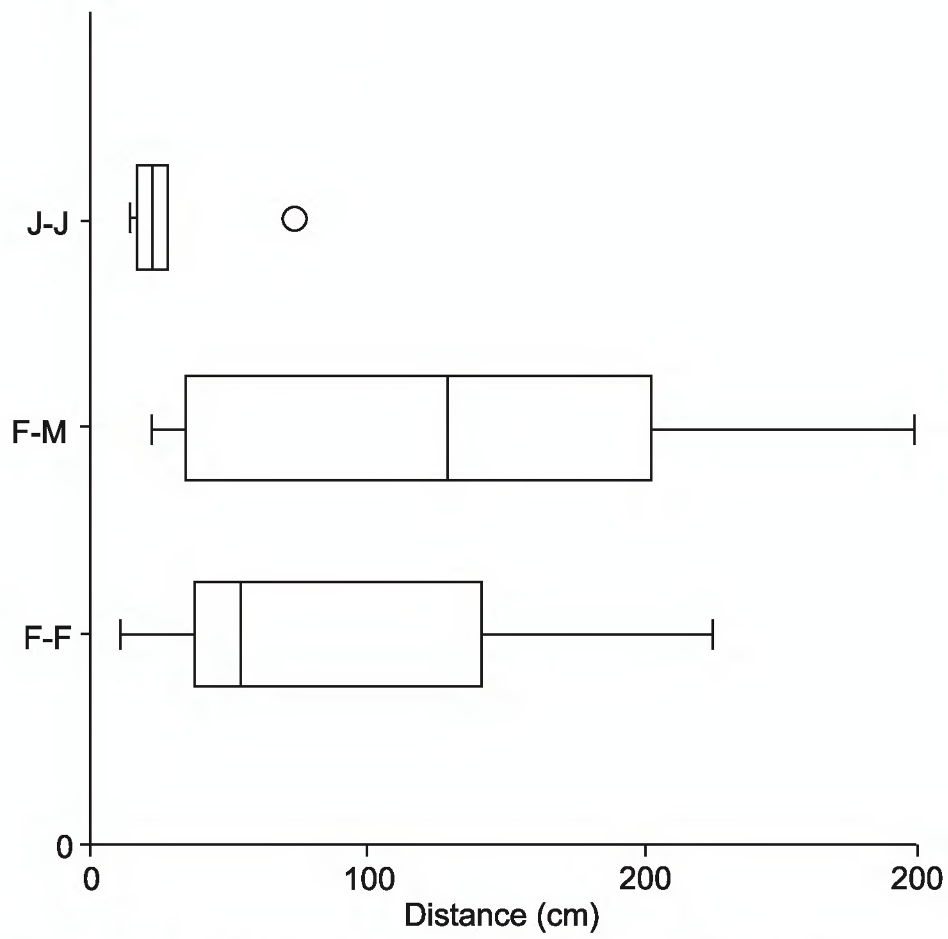

Figure 2. The distance between juveniles to juveniles (J-J), females to males (F-M) and female to female (F-F) conspecifcs of *Charinus asturius*. See text for more details. Vertical bars correspond to the median, whiskers indicate 1.5 times the interquartile range of the data (right or left the first and third quartiles), The point is an outlier.

Figure 3. The whip spider *Charinus asturius* (left side, carapace length ~ 4 mm) and a recluse spider *Loxosceles* sp. (right side) sharing the undersurface of a rock.

We found a female biased Adult Sex Ratio (ASR) in our samples close to 4:1. Twenty-nine of the individuals found were females, seven were males and 26 were juveniles. Among them, some individuals were found under the same rock. In five different times a female was found with a juvenile; we once found a female with two juveniles and a male with two juveniles.

DISCUSSION

We have found that *C. asturius* occur more often on rocks with larger areas close to the substrate, which probably offer more stable microclimatic conditions (see Webb and Shine 1998 and Ferreira and Silva 2001) and protection against predators. Dias and Machado (2006) have shown that the whip spider *Heterophrynus longicornis* (Butler, 1973) is more often found on trees with greater diameter. Hebets (2002) showed a positive correlation between the tree circumference and the total number of individuals of *Phrynus parvulus* Pocock, 1902 found on the trees. Dias and Machado (2006) suggested that greater areas of buttressing could potentially provide an arena for courting, which seems reasonable for our results as well. Therefore, our study shows a new example of preference for specific features of microhabitat in whip spiders.

We did not encounter adults of *C. asturius* of the same sex sheltering under the same rock. The distance between adults is greater than the distance found between juveniles. These findings may be related to territorialism and aggressive behaviors toward conspecifics, which are common in some whip spiders (Weygoldt 2000, Chapin and Hebets 2016, Chapin and Hill-Lindsay 2016). For instance, females of *C. asturius* can kill and cannibalize each other in laboratory (Pinto-da-Rocha et al. 2002). Nonetheless, we observed many cases in which adults and juveniles were sheltering on the same rocks. These findings can potentially be due to a greater tolerance towards individuals of the same kin, as seen in the phrynichid whip spider *Damon diadema* (Simon, 1876) (Walsh and Rayor 2008); and a general tolerance toward conspecific juveniles, probably because they do not compete for sexual partners. However, since we have no information about kinship or the age at which juveniles of *C. asturius* disperse from their mother's rock, both hypotheses still remain to be tested.

We have found a female biased ASR of approximately 4:1 among adults of *C. asturius*. This ASR deviates from the ASR found in other whip spiders' species. Hebets (2002) have found a male biased ASR of approximate 2:1 in *P. parvulus* and Dias and Machado (2006) also found a male biased ASR of approximately 2.5:1 in *H. longicornis*. The myriad of factors that can generate a biased ASR include a biased sex ratio at birth, an uneven mortality between the sexes in juveniles and adults, sex-differential maturation time, dispersal and migration (see Székely et al. 2014). Hebets (2002) has shown that females of *P. parvulus* exhibit higher shelter fidelity than males. If this is also true for *C. asturius* it might explain the female biased ASR found in this study. However, determining the reason for this biased ASR found in *C. asturius* is out of the scope of this study.

Half of the whip spiders were found on the same rocks seven days after being marked. This result suggests that at least some individuals in a population may exhibit shelter fidelity, though we cannot rule out the possibility that these individuals might not have left the rock at all during this period. However, laboratory data suggest that *C. asturius* individuals leave their shelter daily (Pinto-da-Rocha et al. 2002). Future field studies could focus on resighting individuals multiple times at shorter time scales. We also found two marked individuals of *C. asturius* in the same conglomerate of rocks of the original rock. This can be the result of a temporary shelter shift by the individual (see Hebets et al. 2014) or avoidance of the original rock after our disturbance by moving it.

We found recluse spiders in the same shelter of whip spiders. Because recluse spiders are polyphagous (Fischer et al. 2006) and can even prey upon well-protected armored prey (Segovia et al. 2015), they are candidates to be whip spiders' predators.

We encountered individuals of *C. asturius* in the center of the Island, which is a new occurrence for the species that was believed to inhabit only the Pacuíba Mount (Pinto-da-Rocha et al. 2002). We did not find any individual in Sepituba trail, but we do not rule out the possibility that *C. asturius* may occur also in the south of Ilhabela Island. Finally, we clearly demonstrated the importance of rocks for *C. asturius*, preferably the large ones. Although the Pacuíba Mount has many rocks that are used by the whip spider *C. asturius* as shelters, part of this area is not under the Brazilian law of protection as a conservation area. Because Ilhabela Island is a strongly touristic area, there is a possibility of property speculation, which may threaten the habitat of *C. asturius*. Our findings suggest that the area of Pacuíba Mount, as well as the areas of Jabaquara and Água Branca trails must be protected if we are to protect *C. asturius*, a species endemic to Ilhabela Island.

ACKNOWLEDGEMENTS

We are thankful to G. Murayama e J. Fadil for helping us in the field. We thank the staff of the Parque Estadual de Ilhabela: Agnaldo, Betinho, Bicudo, Ivomar, Marcos, Pernambuco and Esmérida for helping us during the field work; G. Machado for helping us with directions to the Pacuíba Mount; R. Moura for helping us with statistical analysis; and G. Gainett and J. Dias for reviewing a draft of this manuscript. The editor Ricardo Pinto da Rocha and the reviewer Gustavo Miranda provided valuable suggestions to improve this manuscript. We are very grateful to Alex Damico for providing us lodging during the field trip. This study was funded by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) process 2014/19191-3 to JMGS, process 2015/01518-9 to RHW and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, process 133214/2015-0) to LCN.

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Submitted: 23 March 2017
Received in revised form: 15 June 2017
Accepted: 2 August 2017
Editorial responsibility: Ricardo Pinto da Rocha

Author Contributions: JMGS and RHW designed the experiments; JMGS and LCN collected the field data; JMGS analysed the data; JMGS, LCN and RHW wrote the paper.

Competing Interests: The authors have declared that no competing interests exist.